IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1642	**Customer No.: 035811**
Examiner	: Brandon J. Fetterolf	
Serial No.	: 10/740,266	
Filed	: December 18, 2003	
Inventors	: Christian Auclair	Docket No.: 1417-03
	: Valérie Amsellem	
	: Martial Hervy	Confirmation No.: 2270
	: Frédéric Subra	
Title	: PHARMACEUTICAL COMPOSITION FOR THE	
	: DIAGNOSIS, PREVENTION OR TREATMENT	
	: OF A TUMORAL PATHOLOGY COMPRISING	
	: AN AGENT MODULATING THE	
	: POLYMERIZATION STATE OF ACTIN	

DECLARATION OF CELINE BOUQUET

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Céline Bouquet, declare that I reside at 5 allée Boris Vian Appt 161, 94310 Orly, France, that I am thoroughly familiar with the above referenced patent application and the subject described therein;

I graduated with an engineer degree from the Institut National Agronomique Paris-Grignon in 1997 with a specialization in "microbiology and micro-organism genetics, option human health". Then I graduated a Ph. D degree in 2002. I performed my Ph.D at the Gustave Roussy institute in CNRS lab. My work was untitled "Inhibition of the pathological angiogenesis via gene transfer of angiostatic factors conjugated to human serum albumin". I published 15 research articles thanks to my DEA and Ph.D degrees, and to academic collaborations. Since 2002, I have been working with BioAllaince Pharma as a project manager. Between 2002 and 2003, I had worked on the project based on phenotypic tumoral reversion and actin cytoskeleton network disorganization in tumoral cells; the strategy was to transfer of the zyxine gene via a

recombinant adenovirus. Since 2003, I have been working on the gene therapy project of BioAlliance Pharma: gene transfer of an antitumoral and antiangiogenic factor using a plasmid vector. I am a named inventor in one patent application.

I have read and understand the above-identified application. It is my understanding that the claims in this application have been rejected because they allegedly contain subject matter that was not described in the Specification in such a way to enable one skilled in the art to make and/or use the invention. I believe that this application fully enables one skilled in the art to make and use the claimed subject matter.

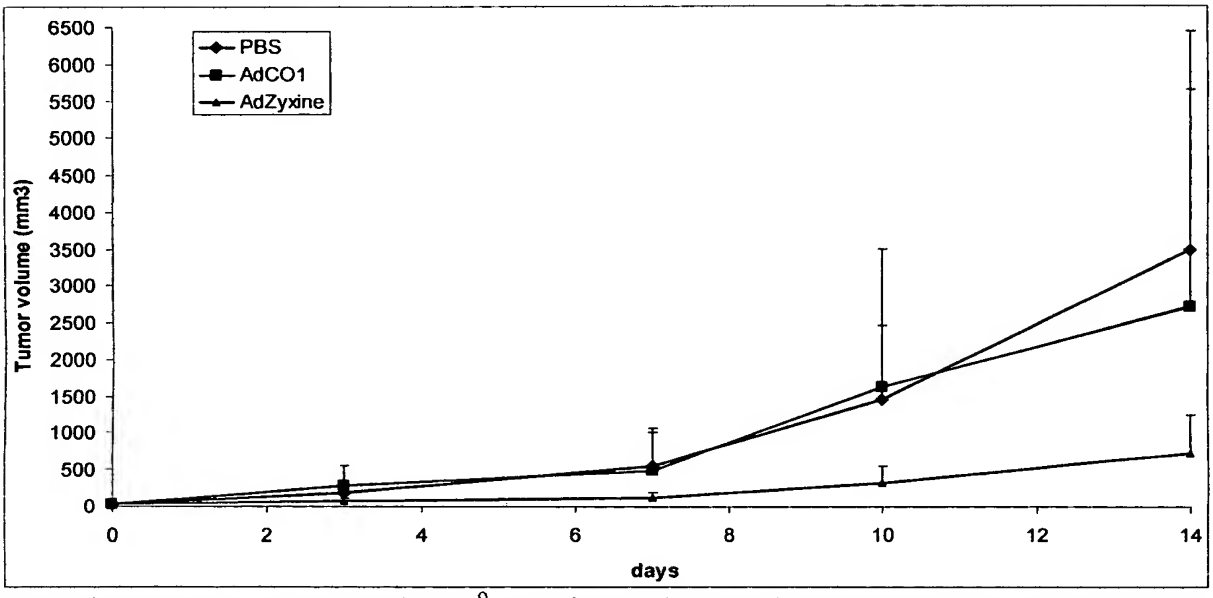
I conducted a series of experiments based on the teachings of the above-identified application and my knowledge in this art and was able to demonstrate the efficacy of the methods of treating. The methodology and results are set forth below.

AdZyxine is a ΔE1–ΔE3 recombinant adenovirus coding for the zyxine gene under the control of the CMV (cytomegalovirus) promoter. Recombinant adenovirus expressing no transgene (AdCO1) was used as a negative control. Recombinant viruses were expanded in 293 cells and purified by a two-step CsCl gradient ultracentrifugation. Viral stocks were desalted using Pharmacia G50 columns (Orsay, France), frozen, and stably preserved at -80°C in PBS containing 10% glycerol. Viral titers were quantified both as pfu/ml following infection of 911 cells and as viral particles (vp)/ml by HPLC analysis.

B16F10 tumor

We injected $2x10^6$ B16F10 murine melanoma cells via the subcutaneous route onto the dorsa of Swiss Nude mice. When mean tumor volume reached $30mm^3$, tumors were intratumorally injected with PBS, or $2x10^9$ pfu of recombinant empty AdCO1 or AdZyxine viruses (in 50µl PBS). Tumor size was monitored by measuring two perpendicular diameters with a digital

caliper. Tumor volume was calculated according to the formula: $(\text{length} + \text{width}/2)^3 \times \pi/6$.

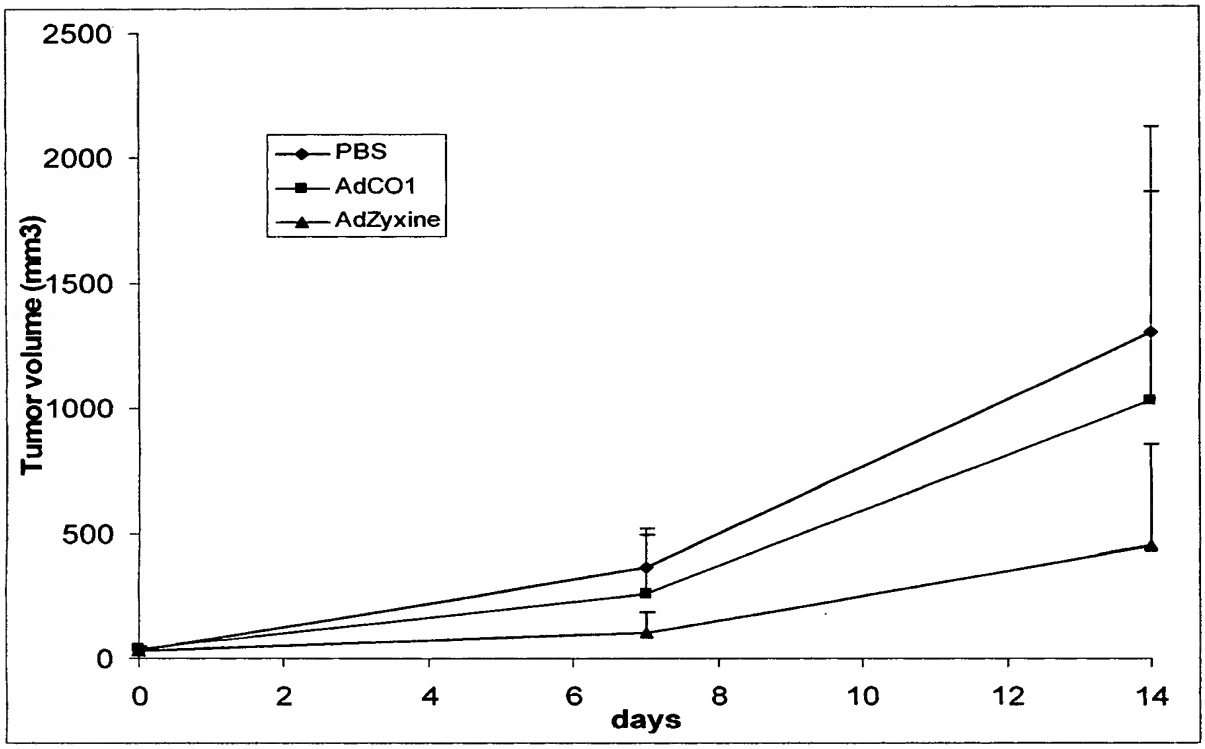


Single intratumoral injection of $2x10^9$ pfu of recombinant adenovirus at day 0. Data represent mean tumor volumes and their standard deviation. PBS n=16, AdCO1 n=16, AdZyxine n=17.

At day 14: 79% of inhibition AdZyxine versus PBS (Student t test p=0.003)
73% of inhibition AdZyxine versus AdCO1 (Student t test p=0.015)

NIH 3T3 EF tumor

We injected $1\text{-}2x10^6$ NIH 3T3 EF cells via the subcutaneous route onto the dorsa of Swiss Nude mice. When mean tumor volume reached $30mm^3$, tumors were intratumorally injected with PBS, or $2x10^9$ pfu of recombinant empty AdCO1 or AdZyxine virus (in 50µl PBS). Tumor size was monitored by measuring two perpendicular diameters with a digital caliper. Tumor volume was calculated according to the formula: $(\text{length} + \text{width}/2)^3 \times \pi/6$.



Single intratumoral injection of $2x10^9$ pfu of recombinant adenovirus at day 0. Data represent mean tumor volumes and their standard deviation. PBS n=7, AdCO1 n=14, AdZyxine n=17.

At day 14: 66% of inhibition AdZyxine versus PBS (Student t test $p<0.001$)
57% of inhibition AdZyxine versus AdCO1 (Student t test $p=0.030$)

The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and thus such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 22th, 2007

Céline Bouquet